

## Stable nitrogen isotope ratios of amino acids reveal the differences in trophic position of benthic fishes in Sendai Bay

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Coastal area provides many ecosystem services such as fishery resources, while it is one of vulnerable ecosystems affected by overfishing, pollution, and development. For the suitable conservation and management, it is necessary to clarify the food web structure and its dynamics. In coastal ecosystem, since some fish species show a widespread migration, their spatio-temporal scales are critical for unraveling the food web dynamics.

Estimation of trophic positions using carbon and nitrogen isotope ratios of bulk tissues are powerful approach for clarifying the food web structure. In the management of fishery resources, trophic positions estimated by stable isotope ratios are regarded as important index of fish resources.

Recent studies reveal that stable nitrogen isotope ratio of individual amino acids ( $\delta^{15}\text{N}_{AA}$ ) is useful for estimating trophic levels (TLs) of organisms. In the amino acid metabolism of organisms, glutamic acid experiences deamination and transamination, which consequences great isotopic enrichment per TL. On the other hand, phenylalanine conserves its amine during metabolism, resulting in little isotopic enrichment per TL. Therefore, the TLs of organisms can be determined by following equation:

$$\text{TL} = (\delta^{15}\text{N}_{Glu} - \delta^{15}\text{N}_{Phe} + \beta) / 7.6 + 1$$

where  $\delta^{15}\text{N}_{Glu}$  and  $\delta^{15}\text{N}_{Phe}$  are stable nitrogen isotope ratios of glutamic acid and phenylalanine of an organism, respectively, and  $\beta$  is the nitrogen isotopic difference between glutamic acid and phenylalanine of a primary producer. For accurate estimation of TL,  $\beta$  values for aquatic and terrestrial primary producers are defined as -3.4 ‰ and +8.4 ‰, respectively.

In this study, we estimated TLs of bastard halibut (*Paralichthys olivaceus*) and stone flounder (*Kareius bicoloratus*) in Sendai Bay using  $\delta^{15}\text{N}_{AA}$  values. After hatch and settling, juvenile fishes inhabit shallow sea areas (water depth <10m), and most of them move to deeper offshore areas with growth. However, some large adult fishes are caught at shallow sea areas.

To reveal the changes of TL with growth and differences of TL among different habitats in these fishes, we collected fish samples from various habitats in Sendai Bay from 2012 to 2014. Amino acids of fish muscle tissues were purified by HCl hydrolysis, followed by N-pivaloyl/isopropyl derivation. The values of  $\delta^{15}\text{N}_{AA}$  were determined by isotope ratio mass spectrometry coupled to a gas chromatograph via combustion and reduction furnaces.

Analysis of  $\delta^{15}\text{N}_{AA}$  clarified increase of TL from juveniles to larger adults of bastard halibut and stone flounder. Furthermore, difference of TL between adult fishes with even body size collected in shallow sea areas and deeper offshore areas suggested that some adult fishes stay at shallow sea areas for a long period. In offshore areas, difference of TL among habitat areas and seasons suggested the variation of food resources. Furthermore, we considered the possibility for tracing the migrations of individual fish using information from  $\delta^{15}\text{N}_{AA}$ .

Keywords: coastal ecosystem, food web, fishery resources, migration tracing